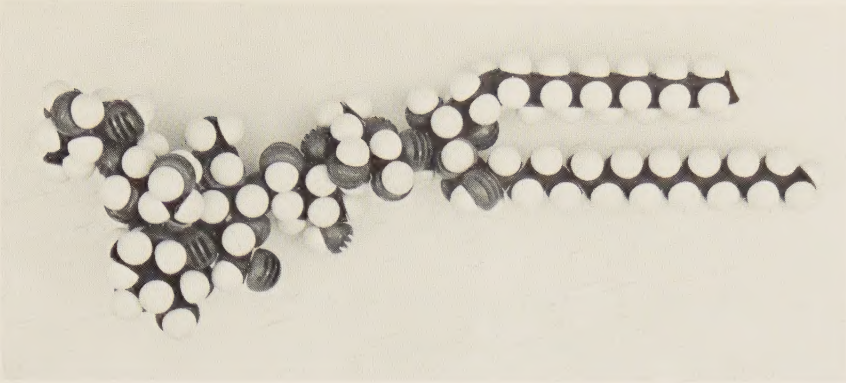


ON THE STRUCTURE AND FUNCTION OF GLYCOSPHINGOLIPIDS

Characterization of blood group antigens of the gastrointestinal tract.

MICHAEL E. BREIMER

Göteborg 1980

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41544702_0008

ON THE STRUCTURE AND FUNCTION OF GLYCOSPHINGOLIPIDS

Characterization of blood group antigens of the gastrointestinal tract.

AKADEMISK AVHANDLING
som, för avläggande av medicine doktorsexamen
vid Göteborgs Universitet, offentligen kommer att försvaras i
Föreläsningssalen, Institutionen för Medicinsk Kemi,
Göteborgs Universitet
kl 09.00 fredagen den 19 december 1980

av
Michael E. Breimer

Göteborg 1980

ABSTRACT

ON THE STRUCTURE AND FUNCTION OF GLYCOSPHINGOLIPIDS

Characterization of blood group antigens of the gastrointestinal tract.

Michael E. Breimer
Department of Medical Biochemistry, University of Göteborg,
Box 33031, S–400 33 Göteborg, Sweden

This thesis is based on the following papers:

I Breimer, M. E., Karlsson, K.-A. and Samuelsson, B. E. Characterization of a Blood Group H Type Pentaglycosylceramide of Human Pancreas Based on a Type 1 Carbohydrate Chain. Submitted.

II Breimer, M. E., Karlsson, K.-A. and Samuelsson, B. E. Characterization of a Human Intestinal Difucosyl Heptaglycolsylceramide with a Blood Group B Determinant and a Type 1 Carbohydrate Chain. *J. Biol. Chem.,* accepted for publication.

III Breimer, M. E., Hansson, G. C., Karlsson, K.-A. and Leffler, H. Demonstration of Complexity of the Glycosphingolipid Fraction of Rat Small Intestine. *Biochem. Biophys. Res. Comm.*, 95 (1980) 416–422.

IV Breimer M. E., Hansson, G. C., Karlsson, K.-A. and Leffler, H. Human Blood Group A-Positive and Negative Strains of Rat. Chemical Basis as Shown by Fucolipids of Small Intestine. *FEBS Lett.,* 114 (1980) 51–56.

V Breimer, M. E. Adaptation of Mass Spectrometry for the Analysis of Tumor Antigens as Applied to Blood Group Glycolipids of a Human Gastric Carcinoma. *Cancer Res.,* 40 (1980) 897–908.

Glandular tissue like pancreas and small intestine is a rich source of blood group active glycosphingolipids compared to parenchymatous organs like liver and kidney. This makes it possible to isolate pure glycolipids in sufficient amounts, even from single individuals, for structural studies. The present thesis deals with the structural characterization of blood group active glycolipids of human pancreas and small intestine, rat small intestine and one case of human gastric adenocarcinoma.

Two novel blood group type glycosphingolipids based on type 1 carbohydrate chain (Galβ1→3GlcNAc) were isolated from human pancreas and small intestine. Structural information about the number, type and sequence of sugars and also ceramide composition was established by mass spectrometry of the intact permethylated and permethylated-reduced derivatives. Further information about the type of sugars was given by gas chromatography after acid degradation of the native glycolipids. The correct sequence and binding positions were established by gas chromatography of partially methylated alditol acetates obtained by degradation of the permethylated and permethylated-reduced glycolipids. Degradation of the permethylated-reduced glycolipids produced disaccharides due to a stabilization of the glycosidic linkage adjacent to the reduced amino group of the amino sugars. The disaccharides were identified by gas chromatography and mass spectrometry. The anomerity of the glycosidic bonds was established by 270 MHz proton NMR spectroscopy of the same derivatives as were used for mass spectrometry. The structure of the pancreas glycolipid was Fuc$\rho\alpha$1 → 2Gal$\rho\beta$1 → 3GlcNAc$\rho\beta$1 → 3Gal$\rho\beta$1 → 4Glc$\rho\beta$1 → 1Cer and the intestinal glycolipid structure was Gal$\rho\alpha$1 → 3Galρ(2 ← 1αFucρ)β1 → 3GlcNAcρ(4← 1αFucρ)β1→3Gal$\rho\beta$1→4Glc$\rho\beta$1→1Cer. The pancreas glycolipid reacted with anti-H antiserum but was less active compared with the type 2 chain (Galβ1→4GlcNAc) isomer of human erythrocytes. The intestinal glycolipid lacked blood group B and Le^b activity.

The total non-acid glycosphingolipid fraction of rat small intestine has been fractionated as acetylated derivative into glycolipid subgroups by silicic acid column chromatography. Thin-layer chromatography revealed a highly complex pattern with at least 30 different glycolipid bands having a thin-layer mobility comparable with mono- to dodecaglycosylceramides. Mass spectrometry of the permethylated and permethylated-reduced ($LiAlH_4$) derivatives showed the presence of several glycolipid species not known before, including oligohexosylceramides with 4, 5, 6 and 7 sugar residues and a tetraglycosylceramide with a blood group A determinant. Glycolipid studies on another rat strain revealed that the glycolipid pattern was similar in both strains except for blood group fucolipids. One strain contained blood group A compounds with 4, 6 and 12 sugar residues while the other strain was A negative and contained blood group H glycolipids with 3, 5 and 10 sugars. These blood group type fucolipids were located exclusively in the epithelial cells of the intestine.

The non-acid glycolipid composition of a gastric adenocarcinoma and the surrounding normal gastric tissue from a human blood group B individual has been analysed. Thin-layer chromatography revealed several differences between the tumor and the normal tissue. Two partially purified tumor fractions were analysed by mass spectrometry. These were chosen on the basis of weak non-compatible blood group A activity. The dominating glycolipids were blood group Le^b– and B-like hexaglycosylceramides, a difucosyl heptaglycosylceramide with a B type saccharide, a H-like heptaglycosylceramide and a Le^b-like octaglycolylceramide. Evidence for a difucosyl heptaglycosylceramide with a blood group A saccharide was also found.

Key words: glycosphingolipid, blood group antigens, small intestine, epithelial cells, mass spectrometry.
ISBN 91-7222-336-7

Department of Medical Biochemistry, University of Göteborg
Göteborg, Sweden

ON THE STRUCTURE AND FUNCTION OF GLYCOSPHINGOLIPIDS

Characterization of blood group antigens of the gastrointestinal tract

by

MICHAEL E. BREIMER

Göteborg 1980

ABSTRACT

ON THE STRUCTURE AND FUNCTION OF GLYCOSPHINGOLIPIDS

Characterization of blood group antigens of the gastrointestinal tract.

Michael E. Breimer
Department of Medical Biochemistry, University of Göteborg,
Box 33031, S–400 33 Göteborg, Sweden

This thesis is based on the following papers:

I Breimer, M. E., Karlsson, K.-A. and Samuelsson, B. E. Characterization of a Blood Group H Type Pentaglycosylceramide of Human Pancreas Based on a Type 1 Carbohydrate Chain. Submitted.

II Breimer, M. E., Karlsson, K.-A. and Samuelsson, B. E. Characterization of a Human Intestinal Difucosyl Heptaglycolsylceramide with a Blood Group B Determinant and a Type 1 Carbohydrate Chain. *J. Biol. Chem.,* accepted for publication.

III Breimer, M. E., Hansson, G. C., Karlsson, K.-A. and Leffler, H. Demonstration of Complexity of the Glycosphingolipid Fraction of Rat Small Intestine. *Biochem. Biophys. Res. Comm.*, 95 (1980) 416–422.

IV Breimer M. E., Hansson, G. C., Karlsson, K.-A. and Leffler, H. Human Blood Group A-Positive and Negative Strains of Rat. Chemical Basis as Shown by Fucolipids of Small Intestine. *FEBS Lett.,* 114 (1980) 51–56.

V Breimer, M. E. Adaptation of Mass Spectrometry for the Analysis of Tumor Antigens as Applied to Blood Group Glycolipids of a Human Gastric Carcinoma. *Cancer Res.,* 40 (1980) 897–908.

Glandular tissue like pancreas and small intestine is a rich source of blood group active glycosphingolipids compared to parenchymatous organs like liver and kidney. This makes it possible to isolate pure glycolipids in sufficient amounts, even from single individuals, for structural studies. The present thesis deals with the structural characterization of blood group active glycolipids of human pancreas and small intestine, rat small intestine and one case of human gastric adenocarcinoma.

Two novel blood group type glycosphingolipids based on type 1 carbohydrate chain (Galβ1→3GlcNAc) were isolated from human pancreas and small intestine. Structural information about the number, type and sequence of sugars and also ceramide composition was established by mass spectrometry of the intact permethylated and permethylated-reduced derivatives. Further information about the type of sugars was given by gas chromatography after acid degradation of the native glycolipids. The correct sequence and binding positions were established by gas chromatography of partially methylated alditol acetates obtained by degradation of the permethylated and permethylated-reduced glycolipids. Degradation of the permethylated-reduced glycolipids produced disaccharides due to a stabilization of the glycosidic linkage adjacent to the reduced amino group of the amino sugars. The disaccharides were identified by gas chromatography and mass spectrometry. The anomerity of the glycosidic bonds was established by 270 MHz proton NMR spectroscopy of the same derivatives as were used for mass spectrometry. The structure of the pancreas glycolipid was Fuc$\rho\alpha$1 → 2Gal$\rho\beta$1 → 3GlcNAc$\rho\beta$1 → 3Gal$\rho\beta$1 → 4Glc$\rho\beta$1 → 1Cer and the intestinal glycolipid structure was Gal$\rho\alpha$1 → 3Galρ(2 ← 1αFucρ)β1 → 3GlcNAcρ(4← 1αFucρ)β1→3Gal$\rho\beta$1→4Glc$\rho\beta$1→1Cer. The pancreas glycolipid reacted with anti-H antiserum but was less active compared with the type 2 chain (Galβ1→4GlcNAc) isomer of human erythrocytes. The intestinal glycolipid lacked blood group B and Le^b activity.

The total non-acid glycosphingolipid fraction of rat small intestine has been fractionated as acetylated derivative into glycolipid subgroups by silicic acid column chromatography. Thin-layer chromatography revealed a highly complex pattern with at least 30 different glycolipid bands having a thin-layer mobility comparable with mono- to dodecaglycosylceramides. Mass spectrometry of the permethylated and permethylated-reduced ($LiAlH_4$) derivatives showed the presence of several glycolipid species not known before, including oligohexosylceramides with 4, 5, 6 and 7 sugar residues and a tetraglycosylceramide with a blood group A determinant. Glycolipid studies on another rat strain revealed that the glycolipid pattern was similar in both strains except for blood group fucolipids. One strain contained blood group A compounds with 4, 6 and 12 sugar residues while the other strain was A negative and contained blood group H glycolipids with 3, 5 and 10 sugars. These blood group type fucolipids were located exclusively in the epithelial cells of the intestine.

The non-acid glycolipid composition of a gastric adenocarcinoma and the surrounding normal gastric tissue from a human blood group B individual has been analysed. Thin-layer chromatography revealed several differences between the tumor and the normal tissue. Two partially purified tumor fractions were analysed by mass spectrometry. These were chosen on the basis of weak non-compatible blood group A activity. The dominating glycolipids were blood group Le^b– and B-like hexaglycosylceramides, a difucosyl heptaglycosylceramide with a B type saccharide, a H-like heptaglycosylceramide and a Le^b-like octaglycolylceramide. Evidence for a difucosyl heptaglycosylceramide with a blood group A saccharide was also found.

Key words: glycosphingolipid, blood group antigens, small intestine, epithelial cells, mass spectrometry.
ISBN 91-7222-336-7

UNIV. LEIDEN BIBL.

The present thesis is based on the following publications, referred to in the text by their Roman numerals.

I Characterization of a Blood Group H Type Pentaglycosylceramide of Human Pancreas Based on a Type 1 Carbohydrate Chain. Submitted. Together with K.-A. Karlsson and B.E. Samuelsson.

II Characterization of a Human Intestinal Difucosyl Heptaglycosylceramide with a Blood Group B Determinant and a Type 1 Carbohydrate Chain. J. Biol. Chem. accepted for publication. Together with K.-A. Karlsson and B.E. Samuelsson.

III Demonstration of Complexity of the Glycosphingolipid Fraction of Rat Small Intestine. Biochem. Biophys. Res. Comm. 95 (1980) 416-422. Together with G.C. Hansson, K.-A. Karlsson and H. Leffler.

IV Human Blood Group A-Positive and -Negative Strains of Rat. Chemical Basis as Shown by Fucolipids of Small Intestine. FEBS Lett. 114 (1980) 51-56. Together with G.C. Hansson, K.-A. Karlsson and H. Leffler.

V Adaptation of Mass Spectrometry for the Analysis of Tumor Antigens as Applied to Blood Group Glycolipids of a Human Gastric Carcinoma. Cancer Res. 40 (1980) 897-908.

CONTENTS

INTRODUCTION	5
Structural variability of glycosphingolipids	5
Distribution of glycosphingolipids	7
Functional aspects on glycosphingolipids	9
Blood group active glycosphingolipids	10
Glycosphingolipid concentration in various tissues	15
Glycosphingolipid methodology	16
PRESENT WORK	18
Structural characterization of blood group glycolipids based on type 1 carbohydrate chain [papers I and II]	19
General characterization of glycosphingolipids of a single organ [paper III]	22
Strain, individual and cell specificity of intestinal blood group glycosphingolipids [paper IV]	23
Glycosphingolipids of a human gastric adenocarcinoma [paper V]	26
REFERENCES	31

INTRODUCTION

The fluid mosaic model of biological membranes proposed by Singer and Nicolson [1] is now accepted as a general model. The membranes are viewed as lipid bilayers in which proteins are dissolved [2]. Fig. 1 shows a schematic model of the plasma membrane. The membrane matrix lipids are asymmetrically distributed [3] with choline phospholipids mainly located in the outer part and the amine phospholipids in the inner layer. Cholesterol is present in both layers. In this lipid bilayer the proteins are interspersed. The carbohydrate chains of glycoproteins and glycolipids are located exclusively on the cell surface.

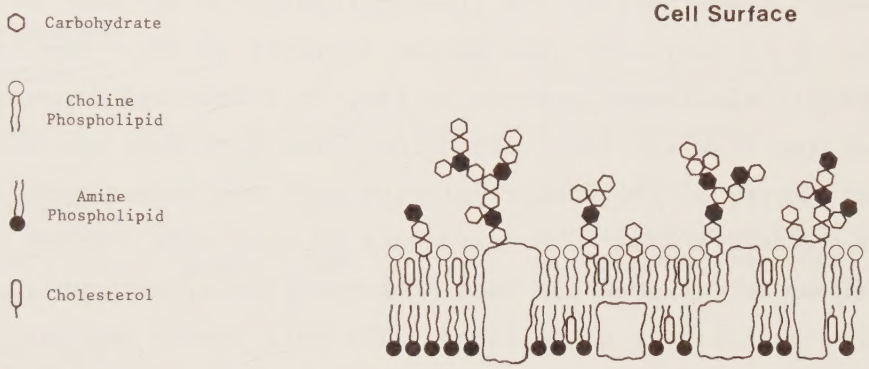


Fig. 1. Schematic model of the plasma membrane structure.

STRUCTURAL VARIABILITY OF GLYCOSPHINGOLIPIDS

Glycosphingolipids contain a long chain base, a fatty acid and carbohydrate. The fatty acid is linked to the long chain base by an amide linkage. This part of the glycolipid is called ceramide. To its primary hydroxyl group the carbohydrate part is bound. The structural formula of a simple glycosphingolipid is shown in Fig. 2.

There is great structural variability among glycosphingolipids. All human long chain bases contain a 1,3-dihydroxy-2-amino structure (sphingosine and related bases), but they differ in carbon atom number (12-22) and in the number, position and geometry of double bonds [4]. There are also bases with an additional hydroxyl group on carbon atom 4 (phytosphingosine and related bases) and there are bases with methyl branches in different positions. Sphingolipid fatty acids contain predominantly 14-26 carbon

atoms and are mainly saturated or monounsaturated [5]. The acids may be non-substituted or substituted with 2-hydroxy groups [6].

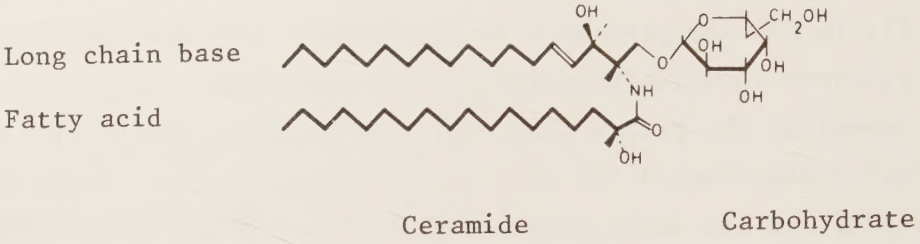


Fig. 2. Structural formula of a monoglycosylceramide. The ceramide part is in this case dihydroxy 18:1 long chain base in combination with 2-hydroxy 18:0 fatty acid. The carbohydrate is galactose.

The carbohydrate part of the glycosphingolipids has been shown to contain from one up to twelve monosaccharide residues [7-10]. The largest glycosphingolipid conclusively shown so far, is a branched dodecaglycosylceramide isolated from rat small intestine. The structure was established by mass spectrometry of the intact permethylated and permethylated-reduced glycolipid derivatives [10]. The existence of even more complex glycolipids with up to 60 sugar residues has been proposed [11-13] but their structures have not been conclusively established. The most common monosaccharides among vertebrate glycosphingolipids are glucose, galactose, fucose, N-acetylglucosamine, N-acetylgalactosamine, N-acetylneuraminic acid and N-glycoloylneuraminic acid [7]. In addition, several other monosaccharides have been found in different glycosphingolipid species isolated from mammals [14-17], invertebrates [18-20], and plants [21-22].

The number of different molecular species of glycolipids is very large. This is due to the variation in carbohydrate sequence, binding position, glycosidic configuration, ring size and branching. And in addition there is also a structural variation in the ceramide part. This structural polymorphism has no correspondence among other lipid groups or polypeptides. Although there are only about 10 common mammalian sugars present, compared with 20 amino acids, the number of possible combinations of sugars is exceedingly large and far above that of amino acids. A calculation of the possible number of disaccharides which can be obtained from 2 different hexoses gives about 70 isomers. This should be compared with the fact that only 2 peptide isomers can be constructed from 2 different amino acids.

The systematization of known glycosphingolipids is based on their

carbohydrate structure. The two major groups are non-acid (neutral) and acid glycolipids. The acid glycosphingolipids contain a negatively charged group and are divided into sulphate-containing glycolipids (sulphatides) and such containing neuraminic acid (gangliosides) [7]. The core structure of the complex glycosphingolipids has been divided into different series in order to make a useful nomenclature system [7,23]. In Table 1 the core structures of some major mammalian glycosphingolipids are listed. There are, however, glycolipids which do not fit into the nomenclature system proposed, for example oligohexosylceramides [paper III] and this may serve as an illustration that a great number of structures are yet unknown.

Table 1. Examples of core structures of some major mammalian glycosphingolipids based on a recent recommendation of glycolipid nomenclature [23].

Glycolipid	Proposed semisynthetic name
Simple members	
Galβ1→1Cer	Galactosylceramide
Glcβ1→1Cer	Glucosylceramide
Galβ1→4Glcβ1→1Cer or Lacβ1→1Cer	Lactosylceramide
Globo series	
Galα1→4Lacβ1→1Cer	Globotriaosylceramide
GalNAcβ1→3Galα1→4Lacβ1→1Cer	Globotetraosylceramide
GalNAcα1→3GalNAcβ1→3Galα1→4Lacβ1→1Cer	Globopentaosylceramide
Isoglobo series	
Galα1→3Lacβ1→1Cer	Isoglobotriaosylceramide
GalNAcβ1→3Galα1→3Lacβ1→1Cer	Isoglobotetraosylceramide
Ganglio series	
GalNAcβ1→4Lacβ1→1Cer	Gangliotriaosylceramide
Galβ1→3GalNAcβ1→4Lacβ1→1Cer	Gangliotetraosylceramide
Lacto series	
GlcNAcβ1→3Lacβ1→1Cer	Lactotriaosylceramide
Galβ1→3GlcNAcβ1→3Lacβ1→1Cer	Lactotetraosylceramide
Lactoneo series	
Galβ1→4GlcNAcβ1→3Lacβ1→1Cer	Lactoneotetraosylceramide

DISTRIBUTION OF GLYCOSPHINGOLIPIDS

Glycosphingolipids have been found in all mammals analysed including man [7,9,24]. They have also been isolated from invertebrates [18,19,25, 26], plants [21,22] and yeast [27]. Glycosphingolipids have not been found in bacteria [28] but have been detected in viruses [29,30] where they are postulated to be localized in the virus envelope which has its origin from

the host cell plasma membrane.

Glycosphingolipid variation related to species, strain and individual. The glycolipid pattern of the same tissue from different animal species shows considerable variation. This is the case for erythrocytes [31], small intestine [32], brain [7,33], retina [33], kidney [7], adipose tissue [34] and other tissues [7,24]. The pattern also differs between different strains of the same animal species such as rat small intestine [paper IV], mouse kidney [35] and erythrocytes [36].

A variation of glycolipids between individuals of the same species also exists due to the genetic regulation of the biosynthesis (see discussion below for the blood group active glycolipids). This is evident for the blood group ABH and Lewis active fucolipids of human [37,38] and dog [39] small intestine and for the P system of erythrocytes [40,41], plasma [41] and gastric tissue [41] of human individuals. A difference also exists for male and female mouse kidney [42] where the male glycolipid pattern could be evoked in the female by testosterone treatment [43].

Glycosphingolipid pattern in different tissues and cells. There is a great difference in glycolipid pattern between the grey and white substance of brain [7,33,44] but even more complex is the difference between neuronal and extra-neuronal glycolipids [7]. The glycolipid pattern of different organs of rat [45,46], chicken [47], bovine [48] and single human individuals [49] varies considerably.

An interesting finding is the variation in glycolipid pattern of different cell types isolated from the same organ. This has been shown for epithelial cells and residual stroma of small intestine [50], T and B lymphocytes [51], and different gastric [52] and brain [7] cell populations.

Subcellular localization. In brain galactosylceramide and sulphatide (galactosylceramide sulphate) are located almost exclusively in the myelin sheet [7,33]. The brain gangliosides are enriched in the synaptosomes and microsomes but there are certain ganglioside species which are only present in the myelin sheet [7,33,44].

For extraneuronal tissue several studies have shown a high concentration of glycosphingolipids in the plasma membrane [7,29,53-57] but others have also noted an intracellular localization [58-61]. When found intracellularly they are usually located in the endoplasmic reticulum and the Golgi apparatus [59,60], from which the surface membranes are postulated to be biosynthetically derived, possibly through vesicle transport and fusion with the surface membrane [62].

In the erythrocyte, which lacks intracellular organelles, the glycolipids have been shown to be located in the outer half of the plasma mem-

brane with their carbohydrate chains exposed to the cell environment [63, 64].

FUNCTIONAL ASPECTS ON GLYCOSPHINGOLIPIDS

The specific physiological function of any particular glycosphingolipid is not known at the present time. However, there are several indications of associations of glycosphingolipids with important cellular functions and in this respect glycolipids differ from other membrane lipid components.

Glycosphingolipids as membrane matrix lipids. If it is assumed that nearly all sphingolipids (glycosphingolipids and sphingomyelin) are located in the outer half of the plasma membrane lipid bilayer, this will mean that about 30-60 % of the lipid molecules in the outer layer must be sphingolipids [37]. In contrast to the glycerophospholipids, which have only hydrogen bond acceptors, sphingolipids have both hydrogen bond acceptors (amide carbonyl) and a variety of donors (hydroxyl) [37,65]. Having this dual character, sphingolipids can make a uniquely ordered region in the surface monolayer of the plasma membrane, due to lateral hydrogen bonds [37, 65]. As a result of this one may predict that an increasing hydroxylation of sphingolipids gives a more dense and impermeable membrane.

Sulphatide and Na^+-K^+ transport across the plasma membrane. Sulphatide has been postulated to be involved in cation transport across the plasma membrane. A cofactor site model was proposed in which sulphatide participates in Na^+ and K^+ discrimination at the plasma membrane as a K^+-selective receptor at the Na^+-K^+-ATPase [37,66].

Glycosphingolipids as cell membrane receptors. Studies in several laboratories have shown that the ganglioside GM_1 is the cell surface receptor for cholera toxin [67-70]. During the last years, several other toxins (tetanus, botulinum), peptide hormones (thyroid-stimulating hormone, human coronic gonadotropin), serotonin, interferon and Sendai virus have been claimed to have gangliosides as cell membrane receptors but there is no conclusive experimental evidence for this at the present time [71-74].

A fucose containing hexaglycosylceramide isolated from rat granuloma and macrophages [75] was shown to be able to interact specifically with MIF (migration inhibition factor) when rat macrophages were examined using a migration inhibition assay system [76]. Recently, another research group has shown in a similar test system, using macrophages from guinea-pig instead of rat, that the MIF receptor may be a ganglioside [77].

Glycolipids of the globo series are able to inhibit the attachment of *E. coli* bacteria to human urinary tract epithelial cells and it was sug-

gested that these glycolipids are the essential cell receptors for uropathogenic *E. coli* bacteria [78,79].

Glycosphingolipids involved in cell-cell recognition phenomena. Glycoproteins and glycolipids have been postulated to participate in cell-cell interaction phenomena like embryonic differentiation [80-84], aggregation of cells in the slime mold *Dictyostelium discoideum* [85,86], cell adhesion [87,88], contact inhibition [89] and malignant transformation [89-92]. Most of the work has been done on carbohydrate binding proteins (lectins), glycosyltransferases and glycoproteins [80,85-88,89-97]. Glycolipid studies on these aspects have been concentrated to their role in malignant transformation of cell cultures [89-92] but some studies on human tumor tissue have also been done [98-102]. Most of the studies on transformed cells and tumor tissue have noted an accumulation of simple glycolipids and a deletion of the more complex ones [89-92]. This simplification of the glycolipid pattern has been largely due to decreased or blocked glycosyltransferase activity instead of a high glycosidase activity [89-92].

Gangliotetraosylceramide was recently shown to be a cell surface marker for natural killer (NK) cells [103] and it has been postulated that this antigen may be involved in the cell-mediated cytotoxicity reaction [104].

Studies on the chicken embryo mesonephros showed that the Forssman antigen, which is a pentaglycosylceramide, was expressed at the surfaces of epithelial cells of the duct and collecting tubules in contrast to its absence from the rest of the nephron [81]. Using monoclonal antibodies, Forssman antigen was found to appear specifically in the early mouse embryogenesis but disappeared later in some cells during the embryo development [84].

BLOOD GROUP ACTIVE GLYCOSPHINGOLIPIDS

The blood group antigens of the ABO, Lewis, P and Ii systems are all built up of carbohydrates [105,106]. There is also evidence that other systems, like M and N, are in part of carbohydrate nature [106]. These carbohydrate antigens are synthesized by a stepwise addition of single sugar units to lipid or protein acceptors [9,71,87,105-107]. Each monosaccharide residue is transferred from a sugar nucleotide donor to the acceptor, and this reaction is catalyzed by a specific glycosyltransferase. The addition of the sugar residues appears to be closely ordered and each glycosyltransferase is highly specific for its sugar nucleotide and acceptor substrate. Each glycosyltransferase is coded by a specific gene but it is not known at the present time how the biosynthesis is regulated.

Blood group ABH and Lewis antigens. The carbohydrate antigens of the

ABO and Lewis systems exist either as free oligosaccharides or bound to proteins or lipids. The free saccharides are present in body fluids like milk [108] and urine [109]. The glycoproteins are either located in the membrane [110] or secreted into various body fluids like milk [108], urine [109], salivia [111] and ovarian cyst fluid [112]. On the other hand, all glycolipid antigens are located in the membrane. Fig. 3 shows the chemical structures and proposed major biosynthetic pathways for the glycosphingolipid blood group ABH and Lewis antigens.

The glycolipid ABH antigens in human tissues are based on lactotetraosylceramide or lactoneotetraosylceramide [9,105,106]. The carbohydrate chains of these two glycolipids are named type 1 and type 2 carbohydrate chains, respectively, which defines the bond between the terminal galactose and the N-acetylglucosamine. Thus Galβ1$\rightarrow$3GlcNAc means a type 1 chain and Galβ1$\rightarrow$4GlcNAc a type 2 chain [9,105].

The addition, by a specific fucosyl transferase, of a fucose residue in an α1$\rightarrow$2 linkage to the terminal galactose of lacto- or lactoneotetraosylceramide produces a blood group H determinant [9,105,106]. The general hypothesis is that the α-2-fucosyl transferase is coded for by the *H* gene which is regulated by the secretor gene (*Se*) in epithelial tissues like submaxillary and breast glands [105]. In haemopoietic tissue the *H* gene is not under control of the *Se* gene [105]. It has recently been postulated that the *H* gene is the structural gene for the α-2-fucosyl transferase acting on type 2 carbohydrate chain and that the *Se* gene is a structural gene for the α-2-fucosyl transferase acting on the type 1 carbohydrate chain [113].

The blood group A and B antigens are formed by addition of N-acetylgalactosamine and galactose, respectively, in an α1$\rightarrow$3 linkage to the subterminal galactose of the blood group H saccharide [9,105,106]. These transferases are coded by the *A* and *B* genes, respectively, and it is believed that these enzymes can act on both type 1 and type 2 chains.

The Lewis gene (*Le*) codes for an α-4-fucosyltransferase which adds a fucose residue to the N-acetylglucosamine of lactotetraosylceramide [9, 105-106] producing the Le^a antigen. If the α-2-fucosyltransferase also acts on this substrate the Le^b antigen is formed. The *Le* enzyme cannot act on lactoneotetraosylceramide [106] but there is obviously an enzyme which can

---

*Fig. 3. Chemical structures and proposed major biosynthetical pathways for the blood group ABH and Lewis antigens based on lactotetraosylceramide (Type 1 chain) and lactoneotetraosylceramide (Type 2 chain). The structures indicated by * were isolated for the first time in this laboratory and the difucosyl compound indicated by ** has not been isolated yet. Only the antigen structures conclusively identified serologically have been named.*

Type 1 chain

(Lactotetraosylceramide)

Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer *

H- gene

Le- gene

Le^a- antigen

Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
4
↑
Fucα1

H- antigen

Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer *
2
↑
Fucα1

Le- gene

Le^b- antigen

Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
2 4
↑ ↑
Fucα1 Fucα1

A- gene

B- gene

A- antigen

GalNAcα1→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
2
↑
Fucα1

Le- gene

GalNAcα1→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer *
2 4
↑ ↑
Fucα1 Fucα1

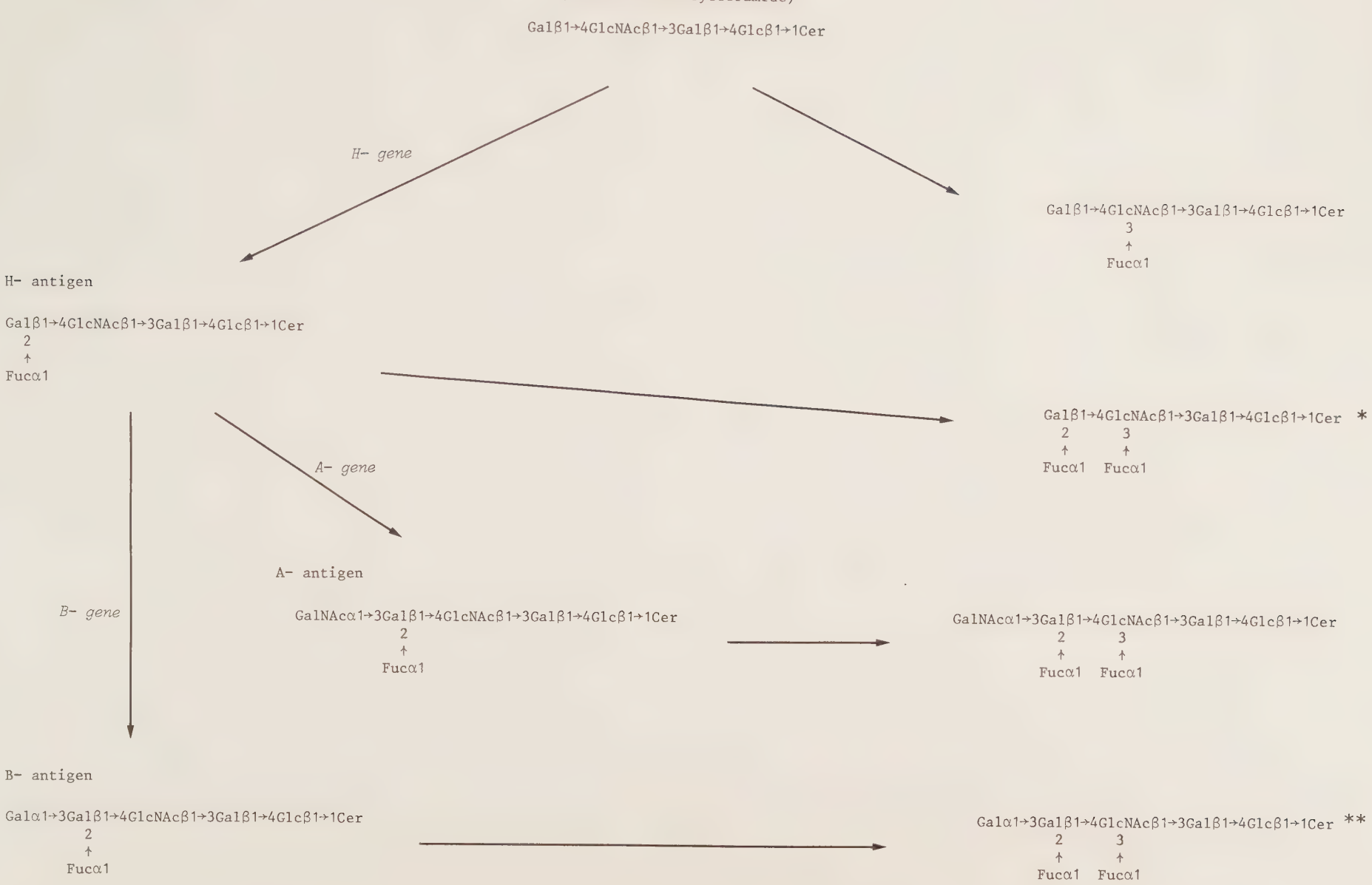
B- antigen

Galα1→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
2
↑
Fucα1

Le- gene

Galα1→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer *
2 4
↑ ↑
Fucα1 Fucα1

Type 2 chain


(Lactoneotetraosylceramide)
Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
H- gene
Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
3
↑
Fucα1
H- antigen
Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
2
↑
Fucα1
Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer *
2 3
↑ ↑
Fucα1 Fucα1
A- gene
A- antigen
GalNAcα1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
2
↑
Fucα1
GalNAcα1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
2 3
↑ ↑
Fucα1 Fucα1
B- gene
B- antigen
Galα1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer
2
↑
Fucα1
Galα1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer **
2 3
↑ ↑
Fucα1 Fucα1

form the type 2 chain isomers of the Le^a and Le^b antigens because these glycolipids have been isolated and structurally characterized [7,9]. The Le^a saccharide isomer was proposed to be named the X antigen and the Le^b isomer the Y antigen [114] and the gene producing the α-3-fucosyltransferase to be named the *X* gene.

The combined action of the *A* gene (or *B*) and the *Le* gene (or *X*) on lactotetraosylceramide (or lactoneotetraosylceramide) results in a hybrid antigen whose structure is a combination of both gene products. Such antigens have been isolated both as oligosaccharides [109] and glycolipids [7, 9, paper II].

The structures of all products which theoretically can be formed by the action of the *A*, *B*, *H*, *Le* and *X* genes on lacto- and lactoneotetraosylceramides are shown in Fig. 3. Their known serological activity is also noted as well as the proposed major biosynthetic pathways. The studies of glycolipid biosynthesis have progressed slowly due to the lack of pure glycolipids as substrates and the difficulties in identification of the products obtained. Most of the classical studies on carbohydrate antigen synthesis have used short saccharides, usually lactose, as substrate [106,115]. So far, only the rat has been shown to contain blood group A, B, H antigens based on lactosylceramide [45,50,116]. One must therefore be careful when these data are used for glycolipid biosynthesis.

The structures shown in Fig. 3 are the basic ones but more complex blood group active glycolipids have been isolated. These include hepta- to decaglycosylceramides with straight carbohydrate chains [7,9,10,50,117, paper V] and branched deca- and dodecaglycosylceramides [7,9,50]. Gangliosides with blood group H determinants are known [118-120] and the type 2 chain isomer of the Le^a glycolipid substituted with sialic acid has been isolated from human kidney [121].

Antigens of the blood group P system. The different antigens of the blood group P system are glycosphingolipids and their chemical structures have been established [122-124]. The structures of the P^k, P and P_1 antigens are listed in Table 2. The P^k (globotriaosylceramide) and P (globotetraosylceramide) antigens are the major glycolipids present in several human tissues like erythrocytes and kidney [5].

Antigens of the blood group Ii system. There is strong evidence that the I and i carbohydrate antigens exist as both glycoprotein [125-127] and glycolipids [11,127-129]. Although there are many different antisera in the Ii system, it is now well established that the majority of blood group i antigenic determinants are expressed on a straight chain carbohydrate sequence and the majority of blood group I determinants are located on

Table 2. Structure of the P^k, P and P_1 glycosphingolipids isolated from human erythrocyte membranes.

P^k	Galα1→4Galβ1→4Glcβ1→1Cer
P	GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer
P_1	Galα1→4Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer

branched structures [127,129]. These two carbohydrate structures are shown in Table 3.

Table 3. Proposed saccharide structures which react with anti-i and anti-I antisera. Different antisera may react with different parts of the antigen.

i	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Gal—
I	Galβ1→4GlcNAcβ1↘ 6 Galβ1→4GlcNAcβ1↗ 3 Galβ1→4GlcNAcβ1→3Gal—

GLYCOSPHINGOLIPID CONCENTRATION IN VARIOUS TISSUES

Glycosphingolipids have been detected in all mammalian tissues characterized so far but the concentration varies considerably [5,7,130]. This is most striking when the white matter of human brain is compared with human erythrocytes. There is about 10^2 times more total glycolipid in the white matter, but for monoglycosylceramides the difference is about 10^4 times [5,7]. In contrast to this, globotetraosylceramide is far more abundant in the erythrocytes [5,7]. Most studies have only dealt with the simple glycolipids having less than 5 sugar residues because these are the major ones in several tissues [5,7]. Structural studies on the complex blood group active fucolipids were first done on human erythrocytes [131,132]. A drawback in these studies was the very low amount of fucolipids. Only a few mg of blood group active fucolipids was obtained from 30 l of blood [133]. Recently it has been shown that the major part (about 75 %) of the blood group active saccharides on the surface of the erythrocytes are bound to protein [134-138] and most of the ceramide linked blood group antigens are present as polyglycosylceramides [11-13].

In contrast to the low amount of fucolipids present in erythrocytes and parenchymatous organs like kidney and liver, the small intestine [32,56,

139-141] and other glandular tissues like pancreas [130,142] are a rich source of blood group active fucolipids. From one single human intestine 31 mg of an Le^a active pentaglycosylceramide was isolated pure [141]. Meconium, which consists of extruded intestinal epithelial cells, contains high amounts of blood group active fucolipids [143].

GLYCOSPHINGOLIPID METHODOLOGY

Preparation of glycosphingolipids. The isolation and structural characterization of glycosphingolipids is often performed in several steps [7-9, 144-147]. The final purification of a glycolipid may be complicated due to the low amounts of these compounds in most tissues and the high complexity of glycolipid species present. Several different preparation methods have been developed [7-9,51,144-147]. These are based on extraction of the tissue with organic solvent, partition and column chromatography using different stationary phases like silicic acid, magnesium silicate, DEAE-cellulose or Sephadex. The use of acetylated derivatives [148] is of great help in removing phospholipids. The final separation of each glycolipid species is done by repeated column chromatography and/or preparative thin-layer chromatography [9].

The purity of the glycolipid fraction is usually checked by thin-layer chromatography [7,8]. It is important that several different solvent systems are used to rule out the possibility of contaminants. It has been shown that one single spot can contain more than one glycolipid component with different carbohydrate structures [149,150]. Further information of the purity is achieved by the use of acetylated derivatives [151,152]. The acetylation may change the relative chromatographic mobility of some glycolipids [papers I and III] which facilitates the preparation of these compounds.

Structural characterization of glycosphingolipids. The classical way to elucidate the structure of a glycolipid is based on the following major methods. The constituent sugars and their molar ratios are established by acid degradation and gas chromatography of the products either as trimethylsilyl derivatives [153] or as alditol acetates [99]. The localization of the glycosidic bonds between the sugars are established by acid degradation of the permethylated glycolipid and analysis of partially methylated alditol acetates by gas chromatography [152,154,155]. The sugar sequence and the anomerity of the glycosidic linkages are analysed by sequential degradation with exoglycosidases and identification of the liberated sugar(s) and the lipid product [9]. Further information can be obtained by partial acid hydrolysis, periodate oxidation and Smith degradation [9]. The lipophilic

part is analysed by acid degradation and gas chromatography of the long-chain bases and fatty acids after suitable derivatization [7].

The major drawback with these methods are the chemical changes of monosaccharides resulting in low recoveries especially concerning amino sugars [154]. This fact may be illustrated by the discussion about the Forssman antigen structure which was not settled until 1973 [154,156] after more than 10 years' work. The conclusive evidence was based on a new technique for analysis of intact glycolipids by mass spectrometry [156,157]. The need for more precise structural methods was obvious when more complex glycolipids with branches were found [152,158] in small amounts, not sufficient for extensive degradation studies.

The long tradition of mass spectrometry and the existence of instrumental facilities at this department due to the late Professor Einar Stenhagen made it natural to develop mass spectrometry for analysis of intact glycolipids. Acetyl and trimethylsilyl derivatives were first used [14, 159-164], but the upper limit for these derivatives was 3 sugar residues. In case of acetylated derivatives this was probably due to pyrolysis at the high temperature needed to get the substrate into gas phase. In order to increase the range of analysis the glycolipids were permethylated according to the method of Hakomori [165] which gives increased volatility with minimal mass increase. Furthermore, the reduction of the amide groups of both the amino sugars and the ceramide by $LiAlH_4$ imposes a stabilization on the molecules [166,167]. The first results were promising [157,166,167] and it is now possible to get the sequence of glycolipids with up to 12 sugar residues [10]. Data about the ceramide composition is also given [168,169]. Mixtures of glycolipids can be analysed [32,150,170] and minor components may be identified by selected ion monitoring [170]. It is also possible to apply this technique for glycopeptide characterization [171]. The method is now used by other research groups [172-174] but presumably the expensive and specialized equipment prevents a more wide-spread use at present.

The anomerity of the glycosidic bonds has usually been established by exoglycosidases [7,9,145]. This method is troublesome to use because the enzymes must be prepared, their specificity tested, and the glycolipid products obtained after incubation must be chemically characterized. Nuclear magnetic resonance (NMR) spectroscopy has not been widely used in glycolipid analysis for several reasons (see [175] for a discussion). In this laboratory proton NMR spectroscopy of permethylated and permethylated-reduced glycolipids has been developed for determining the anomerity of glycolipids with up to 8 sugar residues [175-177]. This method is also applicable on mixtures of glycolipids [50]. Proton NMR spectroscopy of non-derivatized glycolipids in dimethylsulphoxide has recently also been used for glycolipid analysis [178].

The low concentration of glycosphingolipids in most biological tissues is one of the major problems in structural studies of these compounds [5,7, 133]. This is especially the case for the more complex glycolipids having blood group activity [133]. In order to get sufficient material one must usually pool tissue from several individuals which is a great disadvantage due to genetic polymorphism. Most of the structural studies on the blood group ABH and Lewis antigens have been carried out on glycoproteins from milk and ovarian cyst fluid [105,106,179]. From these sources it is possible to get sufficient amounts of glycoprotein from single individuals but a major drawback is that several different carbohydrate chains are attached to each polypeptide backbone which makes the structural studies very complicated [179]. This problem is not present in glycolipid analysis because only one saccharide is bound to ceramide.

When it was noted in the literature that glandular tissue like pancreas was a relatively rich source for blood group fucolipids [130,142] we commenced to pool human pancreas tissue to search for new glycolipid structures. A novel pentaglycosylceramide was easily isolated [paper I] and several other complex glycolipids were found. At this time it was reported that small intestine contained large amounts of blood group fucolipids [139, 140]. In fact, it was possible to isolate pure fucolipids in sufficient amounts for structural characterization from one single human intestine [141]. Therefore our work on pooled tissue was discontinued.

Our first studies on human small intestine were to isolate the total non-acid glycolipid from several intestines [38]. This was done to see if there are differing glycolipid patterns for separate individuals. Fig. 4 shows the thin-layer chromatogram of the total non-acid glycolipids obtained from several human small intestines. For one case (1M) the mucosa was scraped off and analysed [180]. As can be seen there are differences both for individuals of different blood groups and also for individuals with the same blood group. The intestines were obtained at autopsy and routinely typed only for ABO blood groups and therefore the Lewis and secretor status is not known. The glycolipid pattern of the mucosa scraping (Fig. 4, 1M) indicated that the blood group active fucolipids may be exclusively located in the epithelial cells of the intestine [180].

About the same time Weiser had developed a method for sequential removal of intestinal epithelial cells according to their degree of maturation when they migrate from the crypt bottom to the villus tip [181]. With

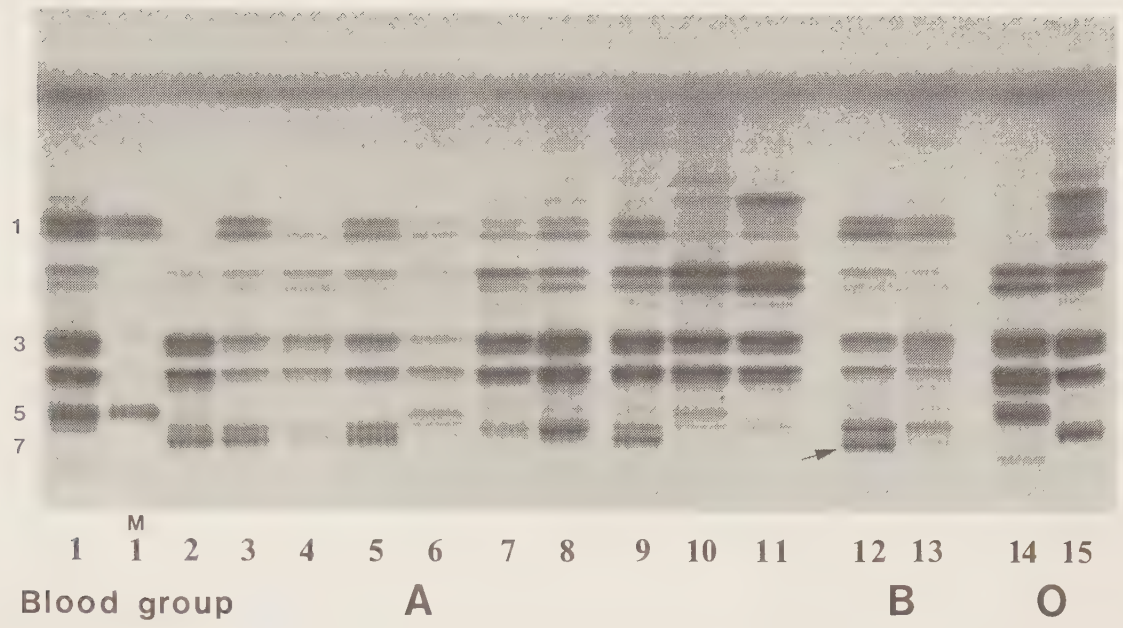


Fig. 4. Thin-layer chromatogram of total non-acid glycosphingolipid fractions isolated from small intestine of human subjects of blood group A (1-11), B (12,13) and 0 (14,15). For intestine 1, M means mucosa scraping. The figures to the left indicate number of sugar residues in each glycolipid band. The compound indicated by an arrow is structurally characterized in paper II. The absence of monoglycosylceramides in cases 2 and 14 is due to an experimental mistake.

this experimental system it may be possible to study changes in glycolipid pattern during a cell differentiation process. In order to get a suitable experimental system to perform these studies we analysed the intestinal glycolipids of several animal species including 2 different strains of rat [32]. The two rat strains were found suitable for further analysis because they had a complex glycolipid pattern with glycolipids containing up to 12 sugar residues [32,50] and were genetically well defined (inbred since 30 years).

STRUCTURAL CHARACTERIZATION OF BLOOD GROUP GLYCOLIPIDS BASED ON TYPE 1 CARBOHYDRATE CHAIN [Papers I and II]

It is important that a glycolipid is isolated pure when its structure is to be solved by conventional structural methods. The use of different solvent systems, stationary phases and chemical derivatives (acetylation, permethylation) helps to rule out the possibility of glycolipid contaminants. The use of mass spectrometry and proton NMR spectroscopy of intact glycolipids has reduced the purity demand because small contaminants are easily detected [paper II]. However, by mass spectrometry of pure glycolipids it

is possible to draw more advanced conclusions about their structure than in the case of mixtures. By use of the specific fragments for type 1 and type 2 carbohydrate chains it was possible to preliminary conclude that the pancreas pentaglycosylceramide and intestinal heptaglycosylceramide were both based on type 1 carbohydrate chain.

Mass spectrometry establishes the exact number of sugar residues of a glycolipid, today up to 12 sugars [10]. This is extremely valuable because the commonly used method for establishing the ratio of sugars is acid degradation-gas chromatography [99] which in our hands has given accurate results only for glycolipids with less than 5 sugar residues.

Gas chromatography of partially methylated alditol acetates obtained by degradation of permethylated glycolipids is used to determine the binding position between the sugar residues [152,154,155]. This method has been further developed in this laboratory by degradation of the permethylated-reduced derivative. The reduction of the permethylated glycolipid with $LiAlH_4$ stabilizes the glycosidic bonds near the amide group of amino sugars giving rise to disaccharides or trisaccharides after acid treatment. The amino sugars of the disaccharides or trisaccharides are in their cyclic form and the hexoses (usually galactose) are reduced to a straight chain by $NaBH_4$. These products have been characterized by mass spectrometry. This reveals the location of the acetyl group on the cyclic amino sugar (type 1 or type 2 chain) and also the location of the bond between the two sugars if NaB^2H_4 is used instead of $NaBH_4$ for reducing the aldehyde group of the hexose. The presence of these disaccharides are in some cases also of help to establish the correct sequence of sugars.

The anomerity of the bonds between the sugar residues was established by 270 MHz proton NMR spectroscopy of permethylated and permethylated-reduced glycolipids [175-177]. The advantages of this technique compared to the use of specific α or β-glycosyl hydrolases are several. The chemical derivatives are the same as used for mass spectrometry and degradative studies and the sample is not consumed during the analysis. The experimental procedure is much simpler because characterization of the degradation products is not needed as for the enzyme method. It is difficult to rule out the presence of small amounts of the other anomer when hydrolases are used and there is always a risk of enzyme impurity. NMR spectroscopy in addition establishes the anomeric structure of the bond between the ceramide and the first sugar in the carbohydrate chain, which is not routinely possible with an enzyme as a commercial preparation is still lacking.

By combined use of these methods the complete structure of the pancreas pentaglycosylceramide was solved. For the intestinal heptaglycosyl-

ceramide, the exact location of the terminal fucose and galactose onto the subterminal galactose was established immunologically by the appearance of blood group B activity after removing the fucose bound to the glucosamine.

Immunological studies of the pancreas pentaglycosylceramide revealed a weaker blood group H activity compared to the type 2 chain isomer isolated from human erythrocytes. In contrast to this, both the type 1 and type 2 chain isomer of blood group A and B hexaglycosylceramides have the same immunological reactivity when tested against anti-A and anti-B antisera [49]. It has been postulated, by studies of free saccharides [182] that the type 1 chain isomer may be the Le^d antigen [182]. It has been shown, both by spectroscopy and in molecular modelling with computer assistance, that the conformation of the type 1 and type 2 chains differ. It was further postulated that a cross reaction of antibodies, directed against blood group H determinants based on these chains, is unlikely [183].

The intestinal heptaglycosylceramide (B-7) is a "hybrid" antigen having both blood group B and Le^b determinants (Fig. 5). It is probably formed by the action of the glycosyltransferase coded by the *Le* gene on the B-6 glycolipid (Fig. 3). This new antigen lacks both blood group B and Le^b activity. This is also the case for the corresponding difucosyl compounds

		Blood group activity	
		B	Le^b
B-7	Galα1→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 2 4 ↑ ↑ Fucα1 Fucα1	-	-
B-6	Galα1→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 2 ↑ Fucα1	+	-
Le^b-6	Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 2 4 ↑ ↑ Fucα1 Fucα1	-	+

Fig. 5. Structural formula and blood group activity of the intestinal difucosyl heptaglycosylceramide (B-7), a blood group B active hexaglycosylceramide (B-6) and a blood group Le^b active hexaglycosylceramide (Le^b-6). All glycolipids are based on type 1 carbohydrate chain.

with blood group A determinant based on both type 1 and type 2 chains [49, 184]. Similar results have been obtained for free oligosaccharides of type 2 chains isolated from human urine [185]. The existence of an antibody which may react with the B-7 compound based on type 1 chain has been reported [186].

Galactosylceramide is the only glycolipid which conformation has been solved by single crystal X-ray analysis [187]. Lemieux et al. have presented conformational formulas for type 1 human blood group oligosaccharides based on ^{13}C and ^{1}H NMR spectroscopy [188]. By use of accurate scale atomic models of the B-7, B-6 and Le^b-6 glycolipids one may propose that the terminal Galα1$\rightarrow$3 of the B-7 glycolipid imposes a steric hindrance for the anti-Le^b antibody. In case of the anti-B antibody this should normally react with more than the terminal trisaccharide as the Fucα1$\rightarrow$4 is able to block binding.

The well-known role of the ABH [189,190] and Lewis [191,192] antigens in the rejection process of kidney transplantation indicates that these difucosyl compounds which are "hybrid" antigens should also be of importance in tissue transplantation.

There is a great need for pure and chemically well defined antigens for different types of immunological experiments such as the search for new antibodies and the production of specific antisera. To establish the dimensions and specificities of recognition sites on lectins and antibodies there is also need for structurally well defined antigens [193].

GENERAL CHARACTERIZATION OF GLYCOSPHINGOLIPIDS OF A SINGLE ORGAN [Paper III]

The aim of this paper was to show (i) the high complexity of glycolipids present in one single organ, (ii) the great advantage of using acetylated glycolipid derivatives for fractionation of the total glycolipid fraction into subgroups, (iii) the use of mass spectrometry for structural characterization of these fractions.

The thin-layer chromatogram of the total non-acid glycolipid fraction obtained from the black and white rat small intestine showed 3 major glycolipid components corresponding to mono-, tri- and tetraglycosylceramides. In addition, several minor components were found. The total fraction was acetylated and fractionated by silicic acid column chromatography. Altogether 7 different fractions were obtained. After deacetylation, the native fractions showed a very complex pattern and about 30 different glycolipid bands were present.

Acetylation influences the relative chromatographic mobility of glycolipids [45,116,194, paper I]. The content of amino sugars plays a major

role for the chromatographic behaviour of the glycolipids as acetylated derivatives. This is most easily seen by the great difference in the Rf values for tetrahexosylceramide and globotetraosylceramide which were the major components of fractions B_4 and B_6. Even small differences in the carbohydrate structure, like a change of linkage position, change the chromatographic mobility [194, paper I]. For some glycolipids the acetylation gives rise to different conformers which may be explained by a steric hindrance of rotation due to the bulky acetyl groups [paper II]. An advantage for the acetylated glycolipids compared to the native ones is that these derivatives are easily solved in organic solvents and do not trail on column chromatography. This can be a major problem for some native compounds [194].

Mass spectrometry of permethylated and permethylated-reduced glycolipid derivatives is a useful technique for characterization of both pure glycolipids [10,168,169] and mixtures [32,170]. The use of mass spectrometry in a primary stage of investigation is informative and consumes very little substance. All 7 glycolipid fractions obtained by fractionation of the acetylated derivatives were analysed by mass spectrometry. This revealed the presence of several glycolipid species not known before, including oligohexosylceramides with 4, 5, 6 and 7 sugar residues and a tetraglycosylceramide with a blood group A determinant. It should be noted that it is possible to analyse the total neutral glycolipid fraction by mass spectrometry without further fractionation which has been done for glycolipids from cat [32] and rabbit [170] intestine. This was done by selected ion monitoring and collection of data by a computer system [32,170]. One problem with analysis of total glycolipid fractions is that minor components may be overshadowed by the major ones. This may be overcome by a prior separation into subgroups.

STRAIN, INDIVIDUAL AND CELL SPECIFICITY OF INTESTINAL BLOOD GROUP GLYCOSPHINGOLIPIDS [Paper IV]

It has been noted that the glycolipid pattern of an organ differs between different strains of the same animal species [35,36]. It was therefore of interest when we chose an experimental animal for epithelial cell studies, to see if there were strain differences in the glycolipid composition of rat small intestine.

The very complex glycolipid pattern found in small intestine of the black and white rat strain [paper III] was also present for the other rat strain (white). The glycolipid composition of the intestine was identical for both strains except for blood group A and H active fucolipids [50].

The black and white strain contained blood group A active tetra-, hexa- and dodecaglycosylceramides which were completely lacking in the white strain. In the white strain blood group H active tri-, penta- and decaglycosylceramides were present. The blood group H active glycolipids are probably the substrate for the glycosyltransferase which adds N-acetyl-galactosamine to form the blood group A determinant. In the black and white strain the H active tri- and pentaglycosylceramides were also present but the decaglycosylceramide appeared to be lacking. This finding indicates that there may be different enzymes for the synthesis of the short and long carbohydrate chains or that the blood group A active dodecaglycosylceramide is synthesized by a multiglycosyltransferase system which does not release the precursor. Other explanations may be that the short and long carbohydrate chain glycolipids are synthesized in different cell compartments or that the enzyme is more effective on longer carbohydrate chains and completely converts the blood group H determinant to A.

The white rat strain is the first blood group A negative strain reported. These two rat strains which differ in their blood group active fucolipids may be of value as an experimental system for studying the role of glycolipids in tissue transplantation.

Earlier work from this laboratory has revealed a specific anatomic localization of glycolipids in small intestine [180]. In a mucosa scraping of human small intestine (Fig. 4) it was shown that monoglycosylceramides and blood group glycolipids with more than 4 sugar residues were confined to the epithelial cells and mono- to tetraglycosylceramides were present in the remaining intestinal stroma [180]. We have modified [195] the technique of Weiser [181] for preparation of intestinal epithelial cells with different maturity. The epithelial cells are completely removed from the intestinal stroma as shown by conventional microscopy [195]. The total non-acid and acidic glycolipid fractions from both the epithelial cells and stroma have been isolated and characterized in detail [50,195] and it was shown that the blood group H and A active glycolipids were exclusively present in the epithelial cells.

We have further developed the technique for isolation of epithelial cells and applied it on human small intestine [195]. Fig. 6 shows the thin-layer chromatogram of total non-acid glycolipids isolated from the epithelial cells (lane E) and from the residual stroma (lane S) of small intestine of a human individual with the serotype A_1Le(a-b+)secretor. The cells contained monoglycosylceramides and complex fucolipids with more than 4 sugar residues while the stroma contained glycolipids of the globo series having up to 4 sugar residues. Monoglycosylceramides were the only species present in both

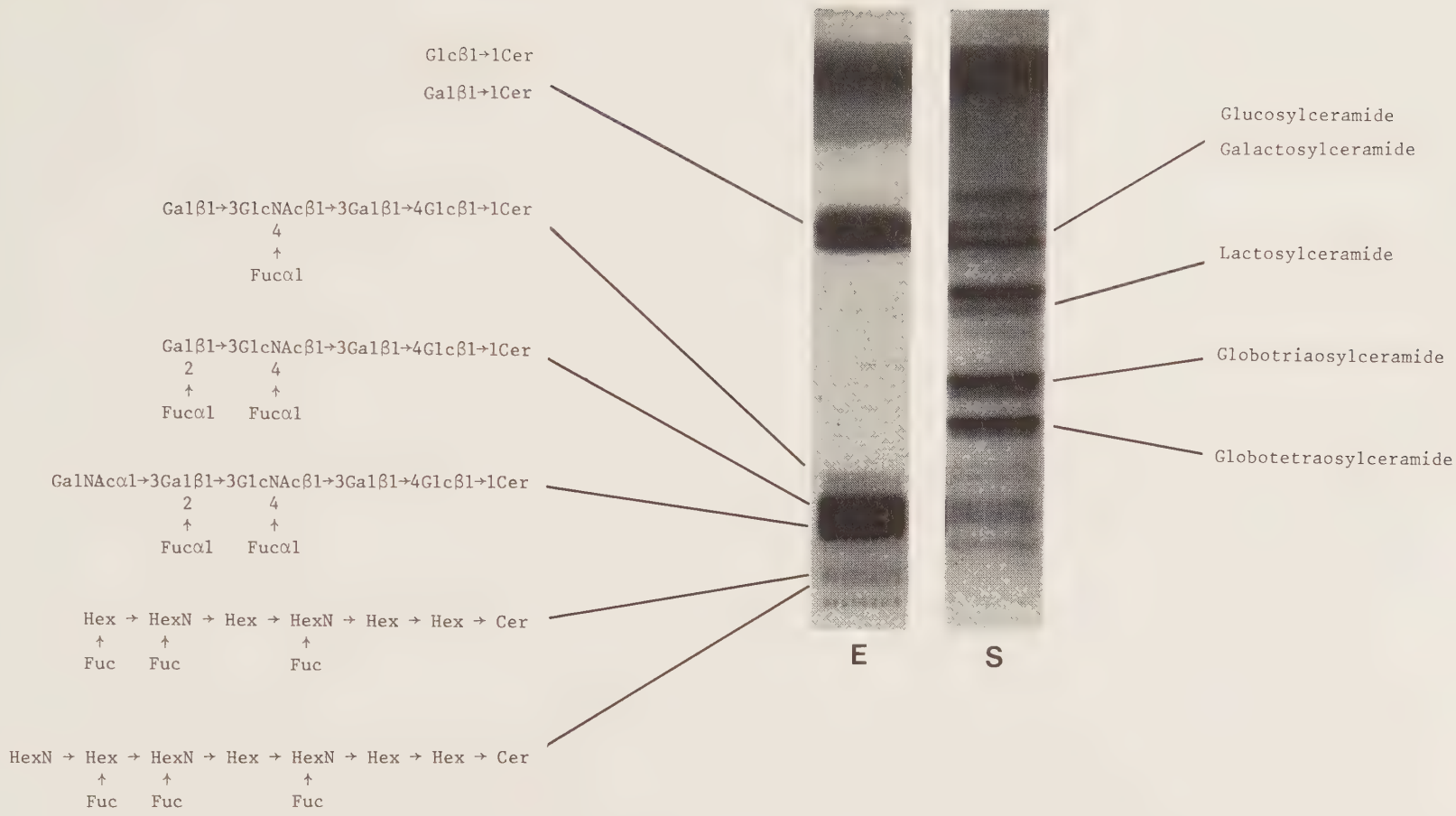


Fig. 6. Thin-layer chromatogram of total non-acid glycolipids isolated from villus epithelial cells (E) and residual stroma (S) of human small intestine. The blood group status of the donor was A_1Le(a-b+)secretor. The amount applied on each lane was 20 microgrammes. The structures of each glycolipid band were established by thin-layer chromatography, mass spectrometry, proton NMR spectroscopy and immunology.

cells and stroma. Further studies on other intestines have revealed a similar distribution of the glycolipids [195]. These results indicate preliminarily that the blood group active fucolipid pattern in the epithelial cells is correlated with the *ABO*, *H*, *Le* and *Se* gene status of the donor [195].

For the case shown in Fig. 6, 11.0 mg and 1.9 mg total non-acid glycolipids were isolated from the epithelial cells and residual stroma, respectively, of a 6 cm long intestinal specimen obtained at surgery. From these figures it can be calculated that a total human small intestine may contain about 700 mg non-acid glycolipids of which 350 mg are complex blood group fucolipids. From the intestines shown in Fig. 4, which were obtained at autopsy, only about 100-200 mg non-acid glycolipids were isolated, probably due to a necrotic loss of villus cells. These extremely high values for blood group fucolipids present in intestinal epithelial cells may be compared with erythrocytes from which only a few mg fucolipids were isolated from more than 30 l blood [133].

The combined use of thin-layer chromatography, mass spectrometry, NMR spectroscopy and immunology makes it possible to do a structural characterization of glycolipid mixtures having only a few milligrammes of substance. This may be of great help for studying animal experimental systems concerned with tumor development, tissue transplantation and the immunological reactions against glycolipid antigens. It is also possible to do a chemical characterization of glycolipids in different pathological diseases in man where only a limited amount of tissue is obtained at surgery or by diagnostic biopsy. Fig. 7 shows a schematic presentation of one possible way to perform a chemical and immunological characterization of glycolipid-based cell surface antigens.

GLYCOSPHINGOLIPIDS OF A HUMAN GASTRIC ADENOCARCINOMA [Paper V]

Most of the work about the role of glycolipids in malignant transformation has been done on cell cultures [89-92]. Some studies of human tumor tissue have also been done [98-102]. Usually these studies have been dealing with the simple glycolipids having less than 5 sugar residues [89-92]. This has been due to the use of partitioning for the removal of polar low-molecular contaminants like salts and amino acids. Therefore the complex polar glycolipids are lost in the upper (water) phase [101]. In those cases where complex glycolipids of human tumor tissue have been studied, usually no comparison with glycolipids of normal tissue of the same individual was done [98-100]. This is desirable because of the individual genetic variation present among the blood group active complex glycolipids.

In the present case the total non-acid glycolipid fraction was ob-

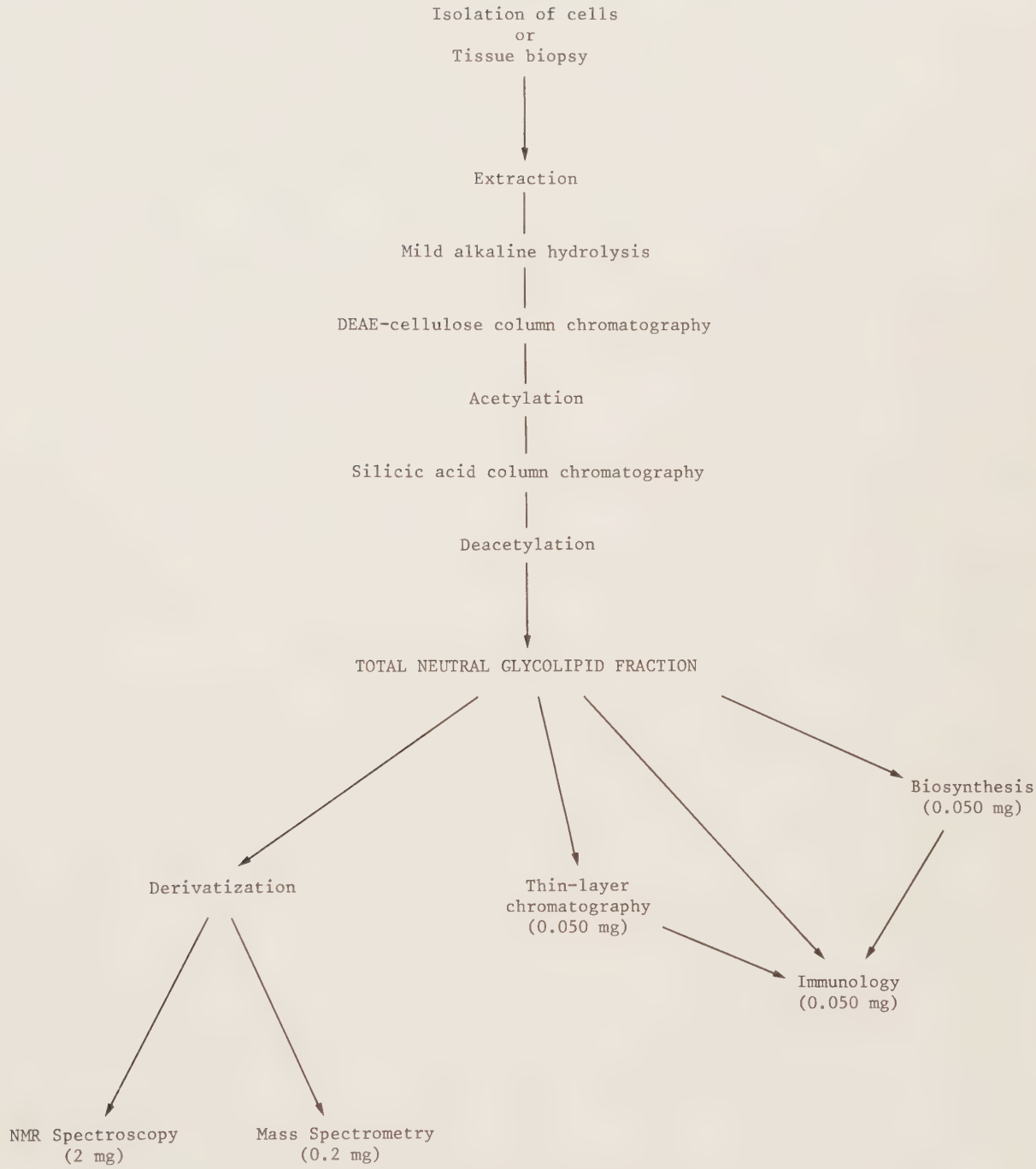


Fig. 7. Schematic presentation of possible ways for chemical and immunological studies of lipid-bound cell surface carbohydrate antigens. The weight figures given indicate the amount of a glycolipid mixture needed for each method.

tained from a gastric adenocarcinoma and the surrounding normal gastric tissue. The weight of the tumor glycolipid fraction was 4 times higher than that of the normal tissue and thin-layer chromatography revealed both qualitative and quantitative differences among the glycolipid species. The total glycolipid fractions were separated into 10 partially purified fractions by silicic acid chromatography. Immunological analysis of these fractions revealed that both the tumor and normal tissue contained blood group B active glycolipids as expected for a B individual. When tested for blood group A activity, two of the tumor fractions but none of the fractions from the normal gastric tissue reacted with the anti-A antisera. These two fractions were analysed by mass spectrometry as their permethylated and permethylated-reduced derivatives. Altogether, 5 major and 3 minor glycolipid species were identified using less than 500 μg of each fraction. The dominating glycosphingolipids were blood group Le^b- and B-like hexaglycosylceramides, a difucosyl heptaglycosylceramide with B-type saccharide, an H-like heptaglycosylceramide, and an Le^b-like octaglycosylceramide. Evidence for small amounts of a blood group H-like pentaglycosylceramide, a difucosyl heptaglycosylceramide with an A-type saccharide and an earlier unknown nonaglycosylceramide with 3 fucoses were also found. This trifucosyl compound may be identical with the similar glycolipid found in human small intestine epithelial cells (Fig. 6) and meconium [196].

The two glycolipid fractions analysed by mass spectrometry were chosen because of their noncompatible blood group activity. Such a noncompatible blood group activity has been documented earlier for gastric and colonic adenocarcinoma by histoimmunological methods [197-198] and in some cases the blood group activity was removed by ethanol treatment of the tissue [198] indicating a glycolipid nature of the antigens. It has been proposed that the blood group A activity in these cases may be a cross reaction with the Forssman antigen [199]. In the present case no Forssman activity was found in the tumor glycolipid fractions. However, our technique has a detection limit of 50 ng compared to the radioimmuno assay technique, using specific anti-Forssman antisera, which is able to detect 1 ng of Forssman antigen [200].

Although the information obtained by mass spectrometry is exact, it is not possible to discriminate stereoisomers and conclusions about the structure of minor components can be difficult to draw because several peaks are overshadowed by fragments from the major glycolipid components. This fact must be borne in mind when postulating the existence of the blood group A-type difucosyl compound in the tumor tissue. Another explanation for the noncompatible serological reaction may be the existence of an anti-

body population in the commercial antiserum which reacts with some other glycolipid present in the tumor fraction.

When the Forssman pentaglycosylceramide was isolated chemically in a human tumor [100] this antigen was the first "tumor specific" antigen structurally characterized because man was regarded as a Forssman negative species. Recent immunological studies have shown that Forssman antigens may be present in both normal gastric tissue and gastric adenocarcinoma [199,200] but a conclusive chemical identification of the Forssman pentaglycosylceramide in a human non-tumor tissue has not been done. Studies on hamster fibroblast NIL cells and polyoma transformed NIL cells (NILpy) revealed that the transformed cells contained lactoneotetraosylceramide which was absent in the normal NIL cells [149]. The accumulation of this glycolipid was proposed to be due to a blocked synthesis of sialyllactoneotetraosylceramide [201]. It was also noted that sera of NILpy tumor-bearing hamsters contained a specific antibody reactive with lactoneotetraosylceramide and that the amount of this antibody in sera was parallel to the size of the tumors [201]. Another study showed that the level of gangliotriaosylceramide in mouse fibroblasts transformed by murine sarcoma virus was greatly increased compared to the non-transformed parental cells [202]. Furthermore, immunization of rabbits with the transformed cells, but not with the normal cells, resulted in production of antibodies against gangliotriaosylceramide [202]. A case report describing the possibility of a successful immunodefense against a noncompatible antigen of the blood group P system in a patient with a gastric carcinoma [203] indicates that the chemical identification of tumor associated antigens may be important for development of immunotherapy as a complement to surgery, radiation and pharmacological therapy.

Most studies examining the glycolipid levels in cultured cells have shown that there is a decrease in more complex glycolipids and a corresponding increase in precursors following oncogenic virus transformation [128-131]. These findings have led to the proposal that tumor cells may have a simplification of their glycolipid composition. This has also been proposed from studies of tumor tissue [89-92,204]. In the present study of a gastric adenocarcinoma the glycolipid pattern of the tumor is even more complex than found for the normal gastric tissue. Until tumors from a variety of tissues and at many stages of differentiation are examined, it appears that generalizations about glycolipid changes in tumors are difficult to make. Studies with transformed cells suggest that simplification as a result of impaired synthesis may be a general rule, but this may not hold for tumors *in situ*. Changes in glycolipid concentrations may be a consequence of the cell type

from which the tumor is derived, the relative density of the cancerous cells or altered metabolic properties of the tumor tissues. Whether glycolipid alterations are in part responsible for tumor behaviour or are a consequence of the tumorgenesis process remains to be determined.

The present study shows that the glycolipid pattern in human tumor tissue is far more complex than earlier known. An enormous work will be needed to establish the exact structure of all glycolipids present both in a tumor and its corresponding normal tissue. Therefore, at the present time one must restrict the use to methods like thin-layer chromatography and mass spectrometry at a primary stage of investigation. A complete structural work that may follow should be applied only in special cases. One way of selection may be the existence of an antiserum reacting specifically with the tumor tissue and a glycolipid prepared thereof.

REFERENCES

1. Singer, S.J. and Nicolson, G.L., Science, 175 (1972) 720-731.
2. Bretscher, M.S., Science, 181 (1973) 622-629.
3. Op den Kamp, J.A., Ann. Rev. Biochem., 48 (1979) 47-71.
4. Karlsson, K.-A., Lipids, 5 (1970) 878-891.
5. Mårtensson, E., Progr. Chem. Fats Lipids, 10 (1969) 367-407.
6. Carter, H.E., Johnson, P. and Weber, E.J., Ann. Rev. Biochem., 34 (1965) 109-142.
7. Sweeley, C.C. and Siddiqui, B., In The Glycoconjugates (Ed. Horowitz, M.I. and Pigman, W.) Academic Press, London, Vol. I (1977) 459-540.
8. Laine, R.A., Steller, K. and Hakomori, S.-I., In Methods in Membrane Biology (Ed. Korn, E.D.) Plenum Press, New York, Vol. 2 (1974) 205-244.
9. McKibbin, J.M., J. Lipid Res., 19 (1978) 131-147.
10. Karlsson, K.-A., In 27th Int. Congress Pure Applied Chem. (Ed. Varmavuori, A.) Pergamon Press, Oxford (1980) 171-183.
11. Kościelak, J., Miller-Podraza, H., Krauze, R. and Piasek, A., Eur. J. Biochem., 71 (1976) 9-18.
12. Gardas, A., Eur. J. Biochem., 68 (1976) 177-183.
13. Dejter-Juszynski, M., Harpaz, N., Flowers, H.M. and Sharon, N., Eur. J. Biochem., 83 (1978) 363-373.
14. Karlsson, K.-A., Samuelsson, B.E. and Steen, G.O., J. Lipid Res., 13 (1972) 169-176.
15. Ishizuka, I., Kloppenburg, M. and Wiegandt, H., Biochim. Biophys. Acta, 210 (1970) 299-305.
16. Hakomori, S.-I. and Saito, T., Biochemistry, 8 (1969) 5082-5088.
17. Buscher, H.-P., Casals-Stenzel, J. and Schauer, R., Eur. J. Biochem., 50 (1974) 71-82.
18. Itasaka, O., J. Biochem., 63 (1968) 347-350.
19. Itasaka, O. and Hori, T., J. Biochem., 85 (1979) 1469-1481.
20. Matsura, F., Chem. Phys. Lipids, 19 (1977) 223-242.
21. Carter, H.E. and Koob, J.L., J. Lipid Res., 10 (1969) 363-369.
22. Hsieh, T.C.-Y., Kaul, K., Laine, R.A. and Lester, R.L., J. Am. Chem. Soc., 17 (1978) 3575-3581.
23. IUPAC-IUB Commission on Biochemical Nomenclature, Eur. J. Biochem., 79 (1977) 11-21.
24. Wiegandt, H., Adv. Lipid Res., 9 (1971) 249-289.
25. Björkman, L.R., Karlsson, K.-A., Pascher, I. and Samuelsson, B.E., Biochim. Biophys. Acta, 270 (1972) 260-265.
26. Karlsson, K.-A., Leffler, H. and Samuelsson, B.E., Biochim. Biophys. Acta, 574 (1979) 79-93.
27. Kaufman, B., Basu, S. and Roseman, S., J. Biol. Chem., 246 (1971) 4266-4271.
28. LaBach, J.P. and White, D.C., J. Lipid Res., 10 (1969) 528-534.
29. Klenk, H.D. and Choppin, P.W., Proc. Natl. Acad. Sci. USA, 66 (1970) 57-64.
30. Diringer, H., Kulas, H.-P., Schneider, L.G. and Schlumberger, H.D., Z. Naturforsch., 28 (1973) 90-93.
31. Yamakawa, T., Irie, R. and Iwanga, M., J. Biochem., 48 (1960) 490-507.
32. Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Leffler, H., Pimlott, W. and Samuelsson, B.E., FEBS Lett., 89 (1978) 42-46.
33. Ledeen, R.W. and Yu, R.K., In Glycolipid Methodology (Ed. Witting, L.A.), Am. Oil Chem. Soc., Champaign, Illinois (1976) 187-214.
34. Ohashi, M., Lipids, 14 (1979) 52-57.
35. Adams, E.P. and Gray, G.M., Chem. Phys. Lipids, 2 (1968) 147-155.
36. Hamanaka, S., Handa, S. and Yamakawa, T., J. Biochem., 86 (1979) 1623-1626.
37. Karlsson, K.-A., In Structure of Biological Membranes (Ed. Abrahamsson, S. and Pascher, I.) Plenum Press, New York (1977) 245-274.

38. Breimer, M.E., Karlsson, K.-A. and Nilsson, K., to be published.
39. Smith, E.L. and McKibbin, J.M., Analyt. Biochem., 45 (1972) 608-616.
40. Kościelak, J., Miller-Podraza, H., Krauze, R. and Cedergren, B., FEBS Lett., 66 (1976) 250-253.
41. Breimer, M.E., Cedergren, B., Karlsson, K.-A., Nilsson, K. and Samuelsson, B.E., FEBS Lett., 118 (1980) 209-211.
42. Coles, L., Hay, J.B. and Gray, G.M., J. Lipid Res., 11 (1970) 158-163.
43. Hay, J.B. and Gray, G.M., Biochim. Biophys. Acta, 202 (1970) 566-568.
44. Ledeen, R.W., Cochran, F.B., Yu, R.K., Samuels, F.G. and Haley, J.E., In Structure and Function of Gangliosides (Ed. Svennerholm, L., Mandel, P., Dreyfus, H. and Urban, P.-F.) Plenum Press, New York (1980) 167-176.
45. Hansson, C.G., Karlsson, K.-A. and Thurin, J., Biochim. Biophys. Acta, (1980) in press.
46. Breimer, M.E., Grip, I., Hansson, G.C., Karlsson, K.-A., Leffler, H. and Thurin, J., to be published.
47. Narasimhan, R. and Murray, R.K., Biochem. J., 173 (1978) 475-482.
48. Breimer, M.E., Karlsson, K.-A. and Samuelsson, B.E., Biochim. Biophys. Acta, 348 (1974) 232-240.
49. Breimer, M.E. and Karlsson, K.-A., to be published.
50. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. and Leffler, H., In Biochemistry of Cell Surface Glycolipids (Ed. Sweeley, C.C.) Am. Chem. Soc. Symposium series 128 (1980) 79-104.
51. Stein, K.E. and Marcus, D.M., Biochemistry, 16 (1977) 5285-5291.
52. Bouhours, J.-F. and Bouhours, D., Biochem. Biophys. Res. Comm., 85 (1978) 1314-1317.
53. Dod, B.J. and Gray, G.M., Biochim. Biophys. Acta, 150 (1968) 397-404.
54. Renkonen, O., Gahmberg, C.G., Simons, K. and Kääriäinen, L., Biochim. Biophys. Acta, 255 (1972) 66-78.
55. Critchley, D.R., Graham, J.M. and McPherson, I., FEBS Lett., 32 (1973) 37-40.
56. Forstner, G.G. and Wherrett, J.R., Biochim. Biophys. Acta, 306 (1973) 446-459.
57. Cooper, J.R. and Kent, P.W., Biochem. Biophys. Res. Comm., 83 (1978) 622-627.
58. Marcus, D.M. and Janis, R., J. Immunol., 104 (1970) 1530-1539.
59. Keenan, T.W., Nyquist, S.E. and Mollenhauer, H.H., Biochim. Biophys. Acta, 270 (1972) 433-443.
60. Keenan, T.W., Morré, D.J. and Huang, C.M., FEBS Lett., 24 (1972) 204-208.
61. Huterer, S. and Wherrett, J.R., Can. J. Biochem., 52 (1974) 507-513.
62. Rothman, J.E. and Fine, R.E., Proc. Natl. Acad. Sci. USA, 77 (1980) 780-784.
63. Steck, T.L. and Dawson, G., J. Biol. Chem., 249 (1974) 2135-2142.
64. Gahmberg, C.G. and Hakomori, S.-I., J. Biol. Chem., 248 (1973) 4311-4317.
65. Abrahamsson, S., Dahlén, B., Löfgren, H., Pascher, I. and Sundell, S., In Structure of Biological Membranes (Ed. Abrahamsson, S. and Pascher, I.) Plenum Press, New York (1977) 1-23.
66. Hansson, G.C., Karlsson, K.-A. and Samuelsson, B.E., J. Biochem., 83 (1978) 813-819.
67. van Heyningen, W.E., Carpenter, C.C.J., Pierce, N.F. and Greenough, W.B., J. Infect. Dis., 124 (1971) 415-418.
68. Cuatrecasas, P., Biochemistry, 12 (1973) 3558-3566.
69. Holmgren, J., Lönnroth, I. and Svennerholm, L., Infection and Immunity, 8 (1973) 208-214.
70. King, C.A. and van Heyningen, W.E., J. Infect. Dis., 127 (1973) 639-647.
71. Fishman, P.H. and Brady, R.O., Science, 194 (1976) 906-915.

72. van Heyningen, S., Biol. Rev., 52 (1977) 509-549.
73. Holmgren, J., Elwing, H., Fredman, P., Strannegård, Ö. and Svennerholm, L., In Structure and Function of Gangliosides (Ed. Svennerholm, L., Mandel, P., Dreyfus, H. and Urban, P.-F.) Plenum Press, New York (1980) 453-470.
74. Kohn, L.D., Lee, G., Grollman, E.F., Ledley, F.D., Mullin, B.R., Friedman, R.M., Meldolesi, M.F. and Aloj, S.M., In Cell Surface Carbohydrate Chemistry (Ed. Harmon, R.E.) Academic Press, New York (1978) 103-133.
75. Hanada, E., Handa, S., Konno, K. and Yamakawa, T., J. Biochem., 83 (1978) 85-90.
76. Miura, T., Handa, S. and Yamakawa, T., J. Biochem., 86 (1979) 773-776.
77. Liu, D.Y., Petschek, K.D., Remold, H.G. and David, J.R., J. Immunol., 124 (1980) 2042-2047.
78. Källenius, G., Möllby, R., Svensson, S.B., Winberg, J., Lundblad, A., Svensson, S. and Cedergren, B., FEMS Microbiol. Lett., 7 (1980) 297-302.
79. Leffler, H. and Svanborg-Edén, C., FEMS Microbiol. Lett., 8 (1980) 127-134.
80. Brownell, A.G., J. Supramolecular Structure, 7 (1977) 223-234.
81. Szulman, A.E., Developmental Biol., 43 (1975) 101-108.
82. Muramatsu, T., Gachelin, G., Nicolas, J.F., Condamine, H., Jakob, H. and Jacob, F., Proc. Natl. Acad. Sci. USA, 75 (1978) 2315-2319.
83. Stern, P.L., Willison, K.R., Lennox, E., Galfré, G., Milstein, C., Secher, D., Ziegler, A. and Springer, T., Cell, 14 (1978) 775-783.
84. Willison, K.R. and Stern, P.L., Cell, 14 (1978) 785-793.
85. Loomis, W.F., Developmental Biol., 70 (1979) 1-12.
86. Ray, J., Shinnick, T. and Lerner, R., Nature, 279 (1979) 215-221.
87. Roseman, S., Chem. Phys. Lipids, 5 (1970) 270-297.
88. Hughes, R.C., In Essays in Biochemistry (Eds. Campbell, P.N. and Aldridge, W.N.) Academic Press, New York, 11 (1975) 1-36.
89. Hakomori, S.-I., In Tumor Lipids: Biochemistry and Metabolism (Ed. Wood, R.) Am. Oil Chem. Soc., Champaign, Illinois (1973) 269-284.
90. Murray, R.K., Yogeeswaran, G., Sheinin, R. and Schimner, B.P., In Tumor Lipids: Biochemistry and Metabolism (Ed. Wood, R.) Am. Oil Chem. Soc., Champaign, Illinois (1973) 285-302.
91. Hakomori, S.-I., Adv. Cancer Res., 18 (1973) 265-315.
92. Hakomori, S.-I., Biochim. Biophys. Acta, 417 (1975) 55-89.
93. Simmons, D.A.R. and Perlman, P., Cancer Res., 33 (1973) 313-322.
94. Kim, Y.S., Isaacs, R. and Perdomo, J.M., Proc. Natl. Acad. Sci. USA, 71 (1974) 4869-4873.
95. Bauer, C.H., Reutter, W.G., Erhart, K.P., Köttgen, E. and Gerok, W., Science, 201 (1978) 1232-1233.
96. Podolsky, D.K. and Weiser, M.M., J. Biol. Chem., 254 (1979) 3983-3990.
97. Chatterjee, S.K., Bhattacharya, M. and Barlow, J.J., Cancer Res., 39 (1979) 1943-1951.
98. Hakomori, S.-I. and Andrews, H.D., Biochim. Biophys. Acta, 202 (1970) 225-228.
99. Yang, H. and Hakomori, S.-I., J. Biol. Chem., 246 (1971) 1192-1200.
100. Kawanami, J., J. Biochem., 72 (1972) 783-785.
101. Karlsson, K.-A., Samuelsson, B.E., Scherstén, T., Steen, G.O. and Wahlquist, L., Biochim. Biophys. Acta, 337 (1974) 349-355.
102. Siddiqui, B., Whitehead, J.S. and Kim, Y.S., J. Biol. Chem., 253 (1978) 2168-2175.
103. Young, W.W., Hakomori, S.-I., Durdik, J.M. and Henney, C.S., J. Immunol, 124 (1980) 199-201.
104. Schwarting, G.A. and Summers, A., J. Immunol., 124 (1980) 1691-1694.
105. Watkins, W.M., Proc. R. Soc. London B, 202 (1978) 31-53.

106. Hakomori, S.-I. and Kobata, A., In The Antigens (Ed. Sela, M.) Academic Press, New York, Vol. II (1974) 79-140.
107. Schachter, H. and Tilley, C.A., In Biochemistry of Carbohydrates II (Ed. Manners, D.J.) University Park Press, Baltimore, Vol. 16 (1978) 209-245.
108. Kobata, A., In The Glycoconjugates (Ed. Horowitz, M.I. and Pigman, W.) Academic Press, London, Vol. I (1977) 423-440.
109. Lundblad, A., In The Glycoconjugates (Ed. Horowitz, M.I. and Pigman, W.) Academic Press, London, Vol. I (1977) 441-458.
110. Winzler, R.J., In Glycoproteins (Ed. Gottschalk, A.) Elsevier Publ. Comp., Amsterdam, Part B (1972) 1268-1293.
111. Mandel, I.D., In The Glycoconjugates (Ed. Horowitz, M.I. and Pigman, W.) Academic Press, London, Vol. I (1977) 153-179.
112. Lloyd, K.O., Kabat, E.A., Layug, E.J. and Gruezo, F., Biochemistry, 5 (1966) 1489-1501.
113. Oriol, R., Personal Communication (1980).
114. Kobata, A., Kagaku, 30 (1975) 192.
115. Ginsburg, V., In Adv. Enzymol. (Ed. Meister, A.) Interscience Publ., New York, Vol. 36 (1972) 131-149.
116. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. and Leffler, H., Biochem. Biophys. Acta, 617 (1980) 85-96.
117. Chien, J.-L., Li, S.-C. and Li, Y.-T., J. Lipid Res. 20 (1979) 669-673.
118. Wiegandt, H., Hoppe-Seyler's Z. Physiol. Chem., 354 (1973) 1049-1056.
119. Suzuki, A., Ishizuka, J. and Yamakawa, T., J. Biochem., 78 (1975) 947-954.
120. Watanabe, K., Powell, M. and Hakomori, S.-I., J. Biol. Chem., 253 (1978) 8962-8967.
121. Rauvala, H., FEBS Lett., 62 (1976) 161-164.
122. Naiki, M. and Marcus, D.M., Biochem. Biophys. Res. Comm., 60 (1974) 1105-1111.
123. Naiki, M., Fong, J., Ledeen, R. and Marcus, D.M., Biochemistry, 14 (1975) 4831-4836.
124. Naiki, M. and Marcus, D.M., Biochemistry, 14 (1975) 4837-4841.
125. Feizi, T. and Kabat, E.A., J. Exp. Med., 135 (1972) 1247-1258.
126. Childs, R.A., Feizi, T., Fukuda, M. and Hakomori, S.-I., Biochem. J., 173 (1978) 333-336.
127. Childs, R.A., Feizi, T. and Tonegawa, Y., Biochem. J., 181 (1979) 533-538.
128. Watanabe, K., Laine, R.A. and Hakomori, S.-I., Biochemistry, 14 (1975) 2725-2733.
129. Watanabe, K., Hakomori, S.-I., Childs, R.A. and Feizi, T., J. Biol. Chem., 254 (1979) 3221-3228.
130. Hakomori, S.-I., Chem. Phys. Lipids, 5 (1970) 96-115.
131. Yamakawa, T. and Iida, T., Japan. J. Exp. Med., 23 (1953) 327-331.
132. Hakomori, S.-I. and Jeanloz, R.W., J. Biol. Chem., 236 (1961) 2827-2834.
133. Hakomori, S.-I. and Strycharz, G.D., Biochemistry, 7 (1968) 1279-1286.
134. Takasaki, S., Yamashita, K. and Kobata, A., J. Biol. Chem., 253 (1978) 6086-6091.
135. Krusius, T., Finne, J. and Rauvala, H., Eur. J. Biochem., 92 (1978) 289-300.
136. Järnefelt, J., Rush, J., Li, Y.-T. and Laine, R.A., J. Biol. Chem., 253 (1978) 8006-8009.
137. Finne, J., Eur. J. Biochem., 104 (1980) 181-189.
138. Wilczyńska, Z., Miller-Podraza, H. and Kościelak, J., FEBS Lett., 112 (1980) 277-279.
139. McKibbin, J.M., Biochemistry, 8 (1969) 679-685.

140. Suzuki, C., Makita, A. and Yosizawa, Z., Arch. Biochem. Biophys., 127 (1968) 140-149.
141. Smith, E.L., McKibbin, J.M., Karlsson, K.-A., Pascher, I., Samuelsson, B.E., Li, Y.-T. and Li, S.-C., J. Biol. Chem., 250 (1975) 6059-6064.
142. Wherrett, J.R. and Hakomori, S.-I., J. Biol. Chem., 248 (1973) 3046-3051.
143. Karlsson, K.-A. and Larson, G., FEBS Lett., 87 (1978) 283-287.
144. Slomiany, A., Slomiany, B.L. and Horowitz, M.I., In Glycolipid Methodology (Ed. Witting, L.A.) Am. Oil Chem. Soc., Champaign, Illinois (1976) 49-75.
145. McKibbin, J.M., In Glycolipid Methodology (Ed. Witting, L.A.) Am. Oil Chem. Soc., Champaign, Illinois (1976) 77-95.
146. Nagai, Y. and Iwamori, M., Molecular and Cellular Biochem., 29 (1980) 81-90.
147. Ando, S. and Yamakawa, T., J. Biochem., 73 (1973) 387-396.
148. Handa, S., Japan. J. Exp. Med., 33 (1963) 347-360.
149. Gahmberg, C.G. and Hakomori, S.-I., J. Biol. Chem., 250 (1975) 2438-2446.
150. Smith, E.L., McKibbin, J.M., Breimer, M.E., Karlsson, K.-A., Pascher, I. and Samuelsson, B.E., Biochim. Biophys. Acta, 398 (1975) 84-91.
151. Kościelak, J., Piasek, A., Gorniak, H., Gardas, A. and Gregor, A., Eur. J. Biochem., 37 (1973) 214-225.
152. Stellner, K., Watanabe, K. and Hakomori, S.-I., Biochemistry, 12 (1973) 656-661.
153. Sweeley, C.C. and Walker, B., Anal. Chem., 36 (1964) 1461-1466.
154. Stellner, K., Saito, H. and Hakomori, S.-I., Arch. Biochem. Biophys., 155 (1973) 464-472.
155. Stoffel, W. and Hanfland, P., Hoppe-Seyler's Z. Physiol. Chem., 354 (1973) 21-31.
156. Karlsson, K.-A., Leffler, H. and Samuelsson, B.E., J. Biol. Chem., 249 (1974) 4819-4823.
157. Karlsson, K.-A., FEBS Lett., 32 (1973) 317-320.
158. Hakomori, S.-I., Stellner, K. and Watanabe, K., Biochem. Biophys. Res. Comm., 49 (1972) 1061-1068.
159. Karlsson, K.-A., Samuelsson, B.E. and Steen, G.O., Biochem. Biophys. Res. Comm., 37 (1969) 22-27.
160. Andersson, B.Å., Karlsson, K.-A., Pascher, I., Samuelsson, B.E. and Steen, G.O., Chem. Phys. Lipids, 9 (1972) 89-111.
161. Karlsson, K.-A., Pascher, I., Samuelsson, B.E. and Steen, G.O., Chem. Phys. Lipids, 9 (1972) 230-246.
162. Karlsson, K.-A., Samuelsson, B.E. and Steen, G.O., Biochim. Biophys. Acta, 306 (1973) 317-328.
163. Sweeley, C.C. and Dawson, G., Biochem. Biophys. Res. Comm., 37 (1969) 6-14.
164. Dawson, G. and Sweeley, C.C., J. Lipid Res., 12 (1971) 56-64.
165. Hakomori, S.-I., J. Biochem., 55 (1964) 205-208.
166. Karlsson, K.-A., Pascher, I., Pimlott, W. and Samuelsson, B.E., Biomed. Mass Spectrom., 1 (1974) 49-56.
167. Karlsson, K.-A., Biochemistry, 13 (1974) 3643-3647.
168. Karlsson, K.-A., In Glycolipid Methodology (Ed. Witting, L.A.) Am. Oil Chem. Soc., Champaign, Illinois (1976) 97-122.
169. Karlsson, K.-A., Progr. Chem. Fats other Lipids, 16 (1977) 207-230.
170. Breimer, M.E., Hansson, G.C., Karlsson, K.-A., Leffler, H., Pimlott, W. and Samuelsson, B.E., Biomed. Mass Spectrom., 6 (1979) 231-241.
171. Karlsson, K.-A., Pascher, I., Samuelsson, B.E., Finne, J., Krusius, T. and Rauvala, H., FEBS Lett., 94 (1978) 413-417.
172. Hanfland, P. and Egge, H., Chem. Phys. Lipids, 15 (1975) 243-247.
173. Ledeen, R.W., Kundu, S.K., Price, H.C. and Fong, J.W., Chem. Phys. Lipids, 13 (1974) 429-446.

174. Watanabe, K., Matsubara, T. and Hakomori, S.-I., J. Biol. Chem., 251 (1976) 2385-2387.
175. Falk, K.-E., Karlsson, K.-A. and Samuelsson, B.E., Arch. Biochem. Biophys., 192 (1979) 164-176.
176. Falk, K.-E., Karlsson, K.-A. and Samuelsson, B.E., Arch. Biochem. Biophys., 192 (1979) 177-190.
177. Falk, K.-E., Karlsson, K.-A. and Samuelsson, B.E., Arch. Biochem. Biophys., 192 (1979) 191-202.
178. Dabrowski, J., Egge, H., Hanfland, P. and Kuhn, S., In Cell Surface Glycolipids (Ed. Sweeley, C.C.) Am. Chem. Soc. Symp. series, 128 (1980) 55-64.
179. Watkins, W.M., Biochem. Soc. Symp., 40 (1974) 125-146.
180. Falk, K.-E., Karlsson, K.-A., Leffler, H. and Samuelsson, B.E., FEBS Lett., 101 (1979) 273-276.
181. Weiser, M.M., J. Biol. Chem., 248 (1973) 2536-2541.
182. Graham, H.A., Hirsch, H.F. and Davies, D.M., In Human Blood Groups (Ed. Mohn, J.F., Plunkett, R.W., Cunningham, R.K. and Lambert, R.M.) S. Karger, Basel (1977) 257-267.
183. Lemieux, R.U., Chem. Soc. Rev., 7 (1978) 423-452.
184. Lloyd, K.O., Kabat, E.A. and Rosenfield, R.E., Biochemistry, 5 (1966) 1502-1507.
185. Lundblad, A. and Kabat, E.A., J. Immunol., 106 (1971) 1572-1577.
186. Jeannet, M., Schapira, M. and Magnin, C., Schweiz. Med. Wschr., 104 (1974) 152.
187. Pascher, I. and Sundell, S., Chem. Phys. Lipids, 20 (1977) 175-191.
188. Lemieux, R.U., Bock, K., Delbaere, L.T.-J., Koto, S. and Rao, V.S., Can. J. Chem., 58 (1980) 631-653.
189. Starzl, T.E., Marchioro, T.L., Holmes, J.H., Hermann, G., Brittain, R.S., Stonington, O.H., Talmage, D.W. and Waddell, W.R., Surgery, 55 (1964) 195-200.
190. Wilbrandt, R., Tung, K.S.K. and Deodhar, S.D., Nakamoto, S. and Kolff, W.J., Am. J. Clin. Pathol., 51 (1969) 15-23.
191. Oriol, R., Cartron, J., Yvart, J., Bedrossian, J., Duboust, A., Bariety, J., Gluckman, J.C. and Gagnadoux, M.F., Lancet I (1978) 574-575.
192. Oriol, R., Opelz, G., Chun, C. and Terasaki, P.I., Transplantation, 29 (1980) 397-400.
193. Kabat, E.A., J. Supramolecular Structure, 8 (1978) 79-88.
194. Karlsson, K.-A. and Larson, G., J. Biol. Chem., 254 (1979) 9311-9316.
195. Breimer, M.E., Hansson, G.C., Karlsson, K.-A. and Leffler, H., to be published.
196. Karlsson, K.-A. and Larson, G., to be published.
197. Häkkinen, I., J. Natl. Cancer Inst., 44 (1970) 1183-1193.
198. Denk, H., Tappeiner, G., Davidovits, A., Eckerstorfer, R. and Holzner, J.H., J. Natl. Cancer Inst., 53 (1974) 933-942.
199. Hakomori, S.-I., Wang, S.-M. and Young, W.W., Proc. Natl. Acad. Sci. USA, 74 (1977) 3023-3027.
200. Young, W.W., Regimbal, J.W. and Hakomori, S.-I., J. Immunological Methods, 28 (1979) 59-69.
201. Sundsmo, J.S. and Hakomori, S.-I., Biochem. Biophys. Res. Comm., 68 (1976) 799-806.
202. Rosenfelder, G., Young, W.W. and Hakomori, S.-I., Cancer Res., 37 (1977) 1333-1339.
203. Levine, P., Ann. N.Y. Acad. Sci., 277 (1976) 428-435.
204. Watanabe, K. and Hakomori, S.-I., J. Exp. Med., 144 (1976) 644-653.

Printed in Sweden, Gotab, Kungälv, 1980 29725